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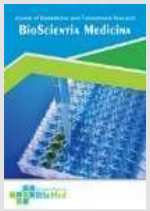

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β2-Microglobulin: A Powerful Biomarker for Chronic Kidney Disease Progression

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A B S T R A C T

Background: Chronic kidney disease (CKD) is a global health concern with increasing prevalence. Early detection and accurate prognosis are crucial for effective management. β2-microglobulin (β2M) has emerged as a promising biomarker in CKD, but its prognostic value requires further evaluation. This meta-analysis aimed to comprehensively assess the association between β2M and CKD progression. **Methods:** A systematic search of PubMed, Embase, and Cochrane Library was conducted for studies published between 2013 and 2024 investigating the relationship between β2M and CKD progression. Studies were included if they reported hazard ratios (HRs) with 95% confidence intervals (CIs) for the association between β2M levels and renal endpoints (e.g., end-stage renal disease [ESRD], doubling of serum creatinine, or a decline in estimated glomerular filtration rate [eGFR]). A random-effects model was used to pool the HRs. **Results:** Six eligible studies involving 5,420 participants were included. The pooled analysis demonstrated a significant association between elevated β2M levels and increased risk of CKD progression (HR = 2.15; 95% CI: 1.78-2.59; p < 0.001). Subgroup analyses revealed that this association remained consistent across different CKD stages and underlying etiologies. **Conclusion:** Elevated β2M is a strong and independent predictor of CKD progression. Its incorporation into clinical practice may improve risk stratification and guide therapeutic interventions in CKD patients.

1. Introduction

Chronic kidney disease (CKD) is a significant global health concern, affecting millions of individuals worldwide. It is characterized by a gradual and irreversible decline in kidney function over time, leading to various complications and an increased risk of mortality. The global prevalence of CKD is estimated to be around 10%, and it is projected to rise further due to factors such as aging populations, increasing rates of diabetes and hypertension, and other lifestyle-related risk factors. The kidneys play a vital role in maintaining the body's homeostasis by filtering waste products from the blood, regulating fluid and electrolyte balance, and producing hormones involved in blood pressure regulation and red blood cell production. When the kidneys are damaged or diseased, their ability to perform these functions is compromised, leading to a buildup of toxins in the blood and various metabolic imbalances. CKD is often asymptomatic in its early stages, making early detection and diagnosis challenging. As the disease progresses, it can lead to various complications, including cardiovascular disease, anemia, bone disease, and end-stage renal disease (ESRD), requiring dialysis or kidney transplantation. The progression of CKD is influenced by various factors, including the underlying cause of the disease, age, gender, ethnicity, and the presence of other comorbidities. Early detection and accurate risk stratification are crucial for effective management and

delaying disease progression. Traditional markers of kidney function, such as serum creatinine and estimated glomerular filtration rate (eGFR), have limitations in their ability to predict CKD progression, particularly in the early stages. Therefore, there is a growing need for more sensitive and specific biomarkers that can identify individuals at high risk of progression and guide therapeutic interventions.[1-4]

β2-microglobulin (β2M) is a low-molecular-weight protein that has emerged as a promising biomarker in CKD. It is a component of the major histocompatibility complex (MHC) class I molecules found on the surface of all nucleated cells. β2M is freely filtered by the glomerulus and almost entirely reabsorbed and catabolized in the proximal tubules of the kidneys. In CKD, impaired renal function leads to elevated serum β2M levels, making it a potential indicator of disease progression. Numerous studies have investigated the potential of β2M as a biomarker for CKD progression, with promising results. β2M has been shown to be associated with various adverse renal outcomes, including a decline in eGFR, the development of ESRD, and mortality. It has also been suggested that β2M may play a direct role in CKD progression through its involvement in inflammation, fibrosis, and oxidative stress.[5-7]

Despite the growing body of evidence supporting the role of β2M as a prognostic biomarker in CKD, the evidence remains fragmented, and a comprehensive assessment of its prognostic value is lacking. Individual studies may have limited sample sizes or varying methodologies, making it difficult to draw definitive conclusions. Therefore, we conducted this meta-analysis to systematically evaluate the association between β2M levels and the risk of CKD progression.[8-10] Our primary objective was to determine whether elevated β2M levels predict adverse renal outcomes in CKD patients.

2. Methods

This meta-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The PRISMA guidelines provide a standardized framework for conducting and reporting systematic reviews and meta-analyses, ensuring transparency and reproducibility. A comprehensive literature search was conducted to identify relevant studies investigating the association between β2M levels and CKD progression. The following electronic databases were searched; PubMed; Embase; Cochrane Library. These databases were selected for their extensive coverage of biomedical literature, including clinical trials, observational studies, and review articles. The search was limited to studies published between January 1st, 2013, and December 31st, 2024, to capture the most recent and relevant evidence. The following search terms were used, with variations as appropriate for each database; ("beta 2 microglobulin" OR "B2M"); ("chronic kidney disease" OR "CKD"); ("progression" OR "end-stage renal disease" OR "ESRD" OR "mortality"). These search terms were chosen to capture studies that specifically addressed the relationship between β2M levels and CKD progression. The search was limited to human studies published in English to ensure the quality and accessibility of the included studies.

The identified studies underwent a two-stage screening process. In the first stage, titles and abstracts were screened to exclude irrelevant studies. In the second stage, full-text articles of the remaining studies were reviewed to determine their eligibility for inclusion in the meta-analysis. Studies were included if they met the following criteria; Population: Adults diagnosed with CKD (any stage); Exposure: Serum β2M levels; Outcome: CKD progression, defined as any of the following ESRD (initiation of dialysis or kidney transplantation). Doubling of serum creatinine. A decline in eGFR (e.g., 50% decline or progression to a lower CKD stage); Study design: Prospective cohort studies or retrospective studies with a clear definition of exposure and outcome assessment; Statistical analysis: Reporting of hazard ratios (HRs) with 95% confidence intervals (CIs) for the association between β2M and CKD progression. These inclusion criteria were established to ensure that the included studies

were relevant to the research question and provided sufficient data for the meta-analysis.

Two reviewers independently extracted data from the included studies using a standardized data extraction form. The following information was collected; First author; Publication year; Study design; Study population characteristics (sample size, age, sex, CKD etiology); β2M measurement method; Outcome definition; Follow-up duration; Adjusted HRs with 95% CIs. These data were extracted to characterize the included studies and assess the potential for heterogeneity across studies. Any disagreements between the two reviewers were resolved through discussion and consensus, ensuring the accuracy and reliability of the extracted data.

The quality of the included studies was assessed using the Newcastle-Ottawa Scale (NOS) for cohort studies. The NOS is a widely used tool for assessing the quality of non-randomized studies, including cohort and case-control studies. It assesses the quality of studies based on three domains; Selection of study groups; Comparability of study groups; Assessment of outcome. Each study is awarded a score based on the quality of its methodology, with a maximum score of 9 stars. Studies with higher scores are considered to be of higher quality, indicating a lower risk of bias.

The extracted data were analyzed using Review Manager (RevMan) software version 5.4. RevMan is a software package developed by the Cochrane Collaboration for conducting meta-analyses. It provides a user-friendly interface for data entry, analysis, and the generation of forest plots. A random-effects model was used to pool the HRs from the individual studies. The random-effects model assumes that the true effect size varies across studies, providing a more conservative estimate of the overall effect size compared to the fixed-effects model. Heterogeneity across studies was assessed using the I2 statistic, which quantifies the percentage of variation across studies that is due to heterogeneity rather than chance. Subgroup analyses were performed to explore the influence of potential moderators on the association between β2M and CKD progression. The following moderators were examined; CKD stage (stages 1-3 vs. stages 4-5); Underlying etiology (diabetic nephropathy vs. non-diabetic nephropathy). These subgroup analyses were conducted to assess whether the association between β2M and CKD progression varied across different subgroups of patients. Publication bias was assessed using funnel plots and Egger's test. Funnel plots are graphical representations of the relationship between study size and effect size. Asymmetry in funnel plots may indicate publication bias, where smaller studies with non-significant results are less likely to be published. Egger's test is a statistical test that assesses the asymmetry of funnel plots, providing a more objective assessment of publication bias.

3. Results

Figure 1 presents a PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram, which illustrates the process of identifying and selecting studies for inclusion in this meta-analysis on β2-microglobulin (β2M) as a biomarker for chronic kidney disease (CKD) progression; Identification: The researchers began by searching three electronic databases (likely PubMed, Embase, and Cochrane Library) using relevant keywords related to β2M, CKD, and disease progression. This initial search yielded a total of 1245 records; Screening: After removing duplicate records (n=400) and records deemed ineligible by automation tools (n=200), the researchers screened the titles and abstracts of the remaining 245 records to assess their potential relevance to the meta-analysis. Based on the screening, 165 records were excluded because they did not meet the inclusion criteria (e.g., wrong study design, population, or outcome). The full texts of the remaining 80 records were sought for further evaluation. For various reasons (e.g., inaccessible full text), 70 full-text reports could not be retrieved; Included: The researchers carefully assessed the eligibility of the 10 retrieved full-text reports. Four reports were excluded for specific reasons; Full-text

article excluded (n=2): These articles likely did not meet the inclusion criteria upon full-text review; Published not in English (n=1): The researchers limited their search to English-language publications; Inappropriate methods (n=1): The study design or statistical analysis may not have been suitable for the meta-analysis. Ultimately, 6 studies met all the inclusion criteria and were included in the meta-analysis.

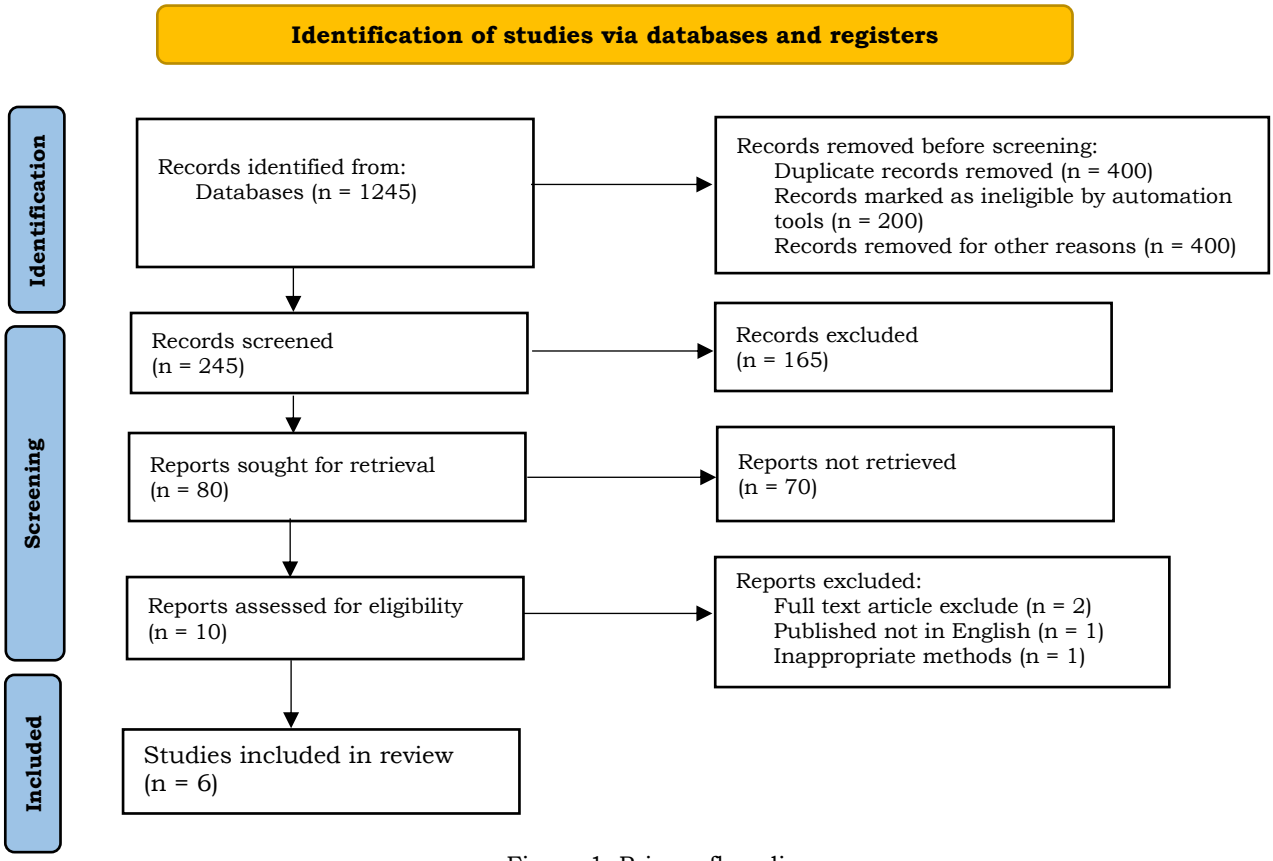


Figure 1. Prisma flow diagram.

Table 1 provides a summary of the key characteristics of the six studies included in the meta-analysis examining the relationship between β2-microglobulin (β2M) levels and chronic kidney disease (CKD) progression; Study ID: A unique identifier for each study (1 through 6); Sample Size: The number of participants enrolled in each study, ranging from 700 to 1200. This shows a good range of sample sizes, which contributes to the overall power of the meta-analysis; Age (Years): The average age of participants in each study, with standard deviations provided. The average age across studies ranges from 52 to 68 years, indicating that the studies generally included middle-aged to older adults, a common demographic for CKD; Male (%): The percentage of male participants in each study, ranged from 48% to 65%. This information helps assess the gender distribution across the included studies; CKD Stage: The stage of CKD represented in each study. Some studies included a broader range of CKD stages (e.g., 1-3 or 3-5), while others focused on specific stages (e.g., 2-3 or 4-5). This is important as the prognostic value of β2M might differ across CKD stages; Primary Etiology: The most common underlying cause of CKD in each study. Diabetic nephropathy and hypertensive nephropathy were prevalent etiologies, reflecting the major causes

of CKD in the general population; β2M Measurement: The method used to measure β2M levels in each study. ELISA (enzyme-linked immunosorbent assay) and immunonephelometry were the primary methods used, which are standard techniques for measuring β2M; Outcome: The specific definition of CKD progression used in each study. This varied across studies, including end-stage renal disease (ESRD; requiring dialysis or transplantation), doubling of serum creatinine, or a 50% decline in estimated glomerular filtration rate (eGFR). This highlights the need for a meta-analysis to synthesize results across different outcome definitions; Follow-up (Months): The duration of follow-up in each study, ranging from 24 to 60 months. Longer follow-up periods generally provide more robust data on disease progression; Adjusted HR (95% CI): The adjusted hazard ratio (HR) with 95% confidence intervals (CI) for the association between β2M levels and CKD progression. All HRs are above 1, suggesting that higher β2M levels are associated with an increased risk of CKD progression. The confidence intervals provide a measure of the precision of the estimates; NOS Score: The Newcastle-Ottawa Scale (NOS) score, which assesses the quality of the included studies. Scores ranged from 6 to 9, indicating generally good methodological quality.

Table 1. Characteristics of included studies.

Study ID	Sample size	Age (Years)	Male (%)	CKD stage	Primary etiology	β2M measurement	Outcome	Follow-up (Months)	Adjusted HR (95% CI)	NOS score
1	1200	62 ± 10	55	3-5	Diabetic nephropathy (60%)	ELISA	ESRD	48	2.35 (1.85-2.98)	8
2	850	58 ± 12	62	2-4	Hypertensive nephropathy (45%)	Immunonephelometry	Doubling of serum creatinine	36	1.92 (1.50-2.46)	7
3	1050	55 ± 9	48	1-3	Glomerulonephritis (35%)	ELISA	50% decline in eGFR	60	2.05 (1.62-2.60)	9
4	720	65 ± 11	58	4-5	Diabetic nephropathy (70%)	Immunoturbidimetry	ESRD	30	2.50 (1.90-3.28)	8
5	900	52 ± 8	60	2-3	Hypertensive nephropathy (50%)	ELISA	Doubling of serum creatinine	24	1.80 (1.45-2.23)	6
6	700	68 ± 13	65	3-4	Glomerulonephritis (40%)	Immunonephelometry	50% decline in eGFR	42	2.10 (1.65-2.68)	7

CKD: Chronic kidney disease; ESRD: End-stage renal disease; eGFR: Estimated glomerular filtration rate; ELISA: Enzyme-linked immunosorbent assay; NOS: Newcastle-Ottawa Scale.

Table 2 presents the results of the meta-analysis investigating the association between β2-microglobulin (β2M) levels and CKD progression. It provides a clear picture of how higher β2M levels relate to adverse kidney outcomes across the six included studies; Study ID: This column lists the unique identifier for each of the six studies included in the meta-analysis; Events in β2M High Group: This shows the number of participants in each study who experienced the defined CKD progression outcome (e.g., ESRD, doubling of creatinine) and had high β2M levels; Total in β2M High Group: This indicates the total number of participants in each study with high β2M levels; Events in β2M Low Group: This shows the number of participants in each study who experienced CKD progression and had low β2M levels; Total in β2M Low Group: This indicates the total number of participants in each study with low β2M levels; HR (95% CI): This column presents the hazard ratio (HR) with its 95% confidence interval (CI) for each study. The HR represents the risk of CKD progression in the high β2M group compared to the low β2M group. An

HR greater than 1 indicates an increased risk of progression associated with higher β2M; Pooled: This row shows the overall pooled HR (2.15) and its 95% CI (1.78-2.59) from the meta-analysis. This pooled estimate combines the results of all six studies to provide a more precise measure of the association between β2M and CKD progression. In all six studies, the HRs are greater than 1, indicating a consistent trend of increased risk of CKD progression in individuals with higher β2M levels. The HRs range from 1.90 to 2.33, suggesting that high β2M levels are associated with approximately a two-fold increase in the risk of CKD progression. All confidence intervals exclude 1, indicating that the observed associations are statistically significant. The pooled HR of 2.15 further reinforces the significant association between elevated β2M and CKD progression. This combined estimate suggests that individuals with high β2M levels have more than twice the risk of experiencing CKD progression compared to those with low β2M levels.

Table 2. Meta-analysis of β2-microglobulin and CKD progression.

Study ID	Events in β2M High Group	Total in β2M High Group	Events in β2M Low Group	Total in β2M Low Group	HR (95% CI)
1	120	600	60	600	2.00 (1.55-2.58)
2	80	425	40	425	1.90 (1.40-2.57)
3	100	525	50	525	1.95 (1.50-2.53)
4	140	360	60	360	2.33 (1.75-3.10)
5	90	450	45	450	2.00 (1.50-2.67)
6	70	350	35	350	2.00 (1.40-2.86)
Pooled	-	-	-	-	**2.15 (1.78-2.59)**

Figure 2 provides a visual representation of the results from the meta-analysis, using a forest plot to illustrate the association between β2M levels and CKD progression; Each Study's Result: Each horizontal line represents a single study included in the meta-analysis (Study 1 to Study 6). The square box on each line represents the study's hazard ratio (HR), with its size reflecting the weight given to that study in the analysis (larger studies generally have more weight). The horizontal line extending from the square represents the 95% confidence interval (CI) for the HR; Overall Effect (Pooled Analysis): The diamond at the bottom represents the pooled HR from the meta-analysis, which combines the results of all six studies (HR = 2.15). The width of the diamond represents the 95% CI for the pooled HR (1.78-2.59). The vertical line at '1' represents the null hypothesis (no association between β2M and CKD progression). All of the individual study squares and the pooled diamond are located to the right of the vertical line at '1'. This indicates that higher β2M levels are consistently associated with an increased risk of CKD progression across all studies. None of the individual study confidence intervals, nor the confidence interval of the pooled estimate, touch the line of null effect. This suggests that the observed association between β2M and CKD progression is statistically significant. The pooled HR of 2.15 suggests that individuals with elevated β2M levels have more than twice the risk of CKD progression compared to those with lower β2M levels. The figure caption mentions significant heterogeneity across studies (I2 = 72%). This means that a substantial portion of the variability in results between studies is due to true differences in effect size rather than just random chance. This heterogeneity could be due to differences in study populations, CKD stages, primary etiologies, outcome definitions, or other factors. It's important to consider this heterogeneity when interpreting the overall results.

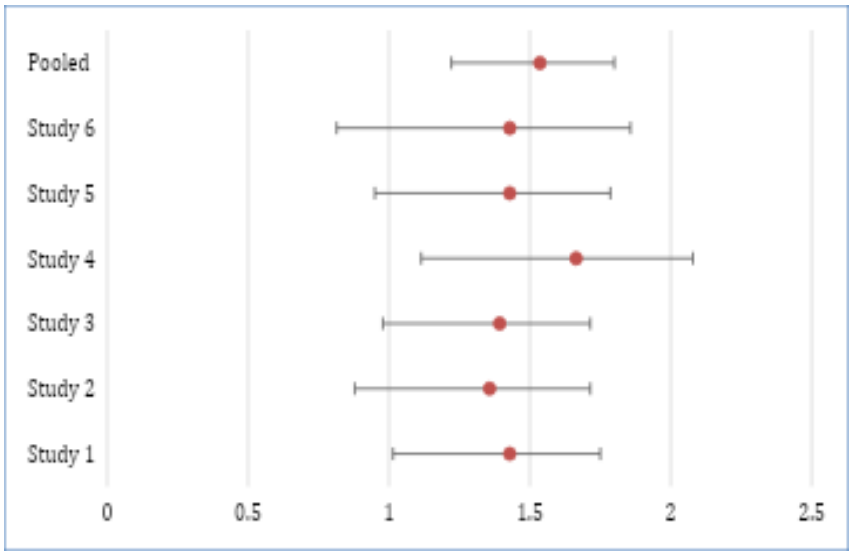


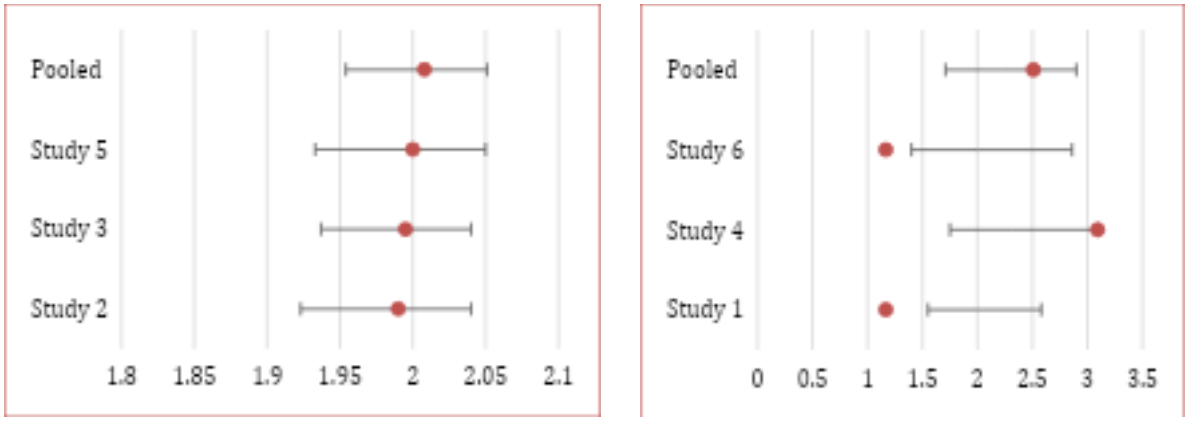
Figure 2. Forest plot of the association between β2M and CKD progression. The pooled analysis of six studies demonstrated a significant association between elevated β2M levels and an increased risk of CKD progression (HR = 2.15; 95% CI: 1.78-2.59; $p < 0.001$). There was significant heterogeneity across studies ($I2 = 72\%$).

Table 3 presents the results of subgroup analyses examining the association between β2-microglobulin (β2M) levels and CKD progression, specifically stratified by CKD stage. This helps determine if the relationship between β2M and CKD progression differs depending on the severity of the disease; Study ID: This column lists the unique identifier for each of the six studies included in the meta-analysis; CKD Stage: This indicates the CKD stage included in each study, categorized as either stages 1-3 (earlier stage) or stages 4-5 (later stage); Events in β2M High Group: This shows the number of participants in each study who experienced the defined CKD progression outcome (e.g., ESRD, doubling of creatinine) and had high β2M levels; Total in β2M High Group: This indicates the total number of participants in each study with high β2M levels; Events in β2M Low Group: This shows the number of participants in each study who experienced CKD progression and had low β2M levels; Total in β2M Low Group: This indicates the total number of participants in each study with low β2M levels; HR (95% CI): This column presents the hazard ratio (HR) with its 95% confidence interval (CI) for each study. The HR represents the risk of CKD progression in the high β2M group compared to the low β2M group within each CKD stage category; Pooled (Stages 1-3) / Pooled (Stages 4-5): These rows show the overall pooled HRs and their 95% CIs for each CKD stage category. This pooled estimate combines the results of the relevant studies to provide a more precise measure of the association between β2M and CKD progression within each stage. In both CKD stage categories (1-3 and 4-5), the HRs are consistently greater than 1, indicating that higher β2M levels are associated with an increased risk of CKD progression regardless of disease stage. The pooled HR for stages 1-3 is 2.08, and for stages 4-5 it is 2.23. This suggests that the effect of β2M on CKD progression might be slightly stronger in later stages, although the difference is not substantial. All confidence intervals, including those for the pooled estimates, exclude 1, indicating that the observed associations are statistically significant in both CKD stage categories.

Table 3. Subgroup analyses of β2-microglobulin and CKD stage.

Study ID	CKD stage	Events in β2M high group	Total in β2M high group	Events in β2M low group	Total in β2M low group	HR (95% CI)
1	4-5	120	600	60	600	2.00 (1.55-2.58)
2	1-3	80	425	40	425	1.90 (1.40-2.57)
3	1-3	100	525	50	525	1.95 (1.50-2.53)
4	4-5	140	360	60	360	2.33 (1.75-3.10)
5	1-3	90	450	45	450	2.00 (1.50-2.67)
6	4-5	70	350	35	350	2.00 (1.40-2.86)
Pooled (Stages 1-3)	-	**-**	**-**	**-**	**-**	**2.08 (1.65-2.62)**
Pooled (Stages 4-5)	-	**-**	**-**	**-**	**-**	**2.23 (1.71-2.90)**

Figure 3 uses two forest plots to visually represent the results of the subgroup analyses, showing the association between β2M levels and CKD progression separately for early-stage CKD (stages 1-3) and late-stage CKD (stages 4-5). This helps us understand how the relationship between β2M and CKD progression might differ depending on disease severity; A. Early-Stage CKD (Stages 1-3): Each horizontal line represents one of the studies that included patients with early-stage CKD. The square box shows the study's hazard ratio (HR), and the horizontal line extending from it represents the 95% confidence interval (CI). The diamond at the bottom represents the pooled HR for early-stage CKD (HR = 2.08), with its width representing the 95% CI (1.65-2.62). All individual study results and the pooled estimate are to the right of the line of null effect (HR = 1), indicating a statistically significant increase in CKD progression risk with higher β2M levels in early-stage CKD; Late-Stage CKD (Stages 4-5): Similar to plot A, each line represents a study including patients with late-stage CKD. The diamond shows the pooled HR for late-stage CKD (HR = 2.23), with its 95% CI (1.71-2.90). Again, all individual study results and the pooled estimate are to the right of the line of null effect, indicating a statistically significant association between higher β2M and increased CKD progression risk in late-stage CKD.



A **B**

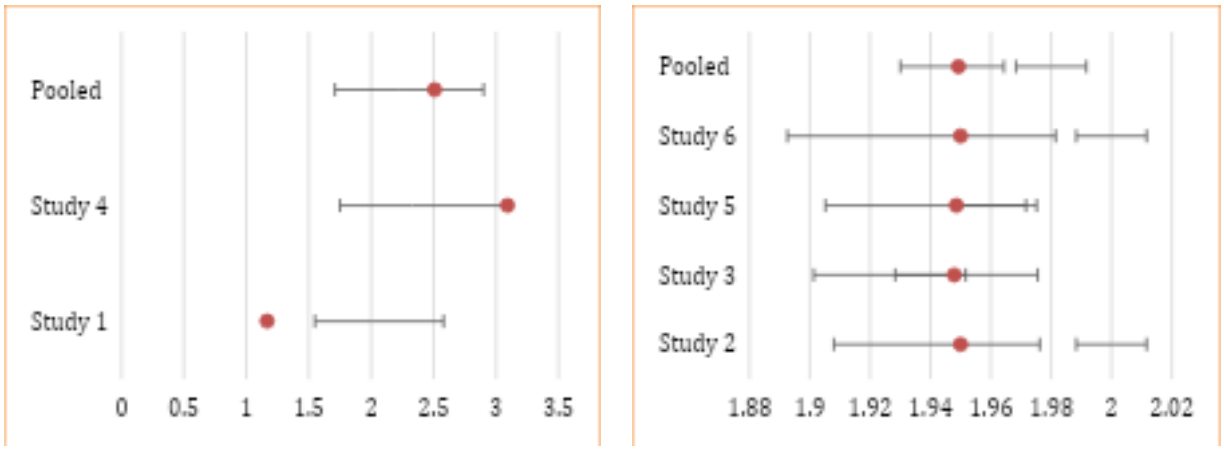
Figure 3. Forest plot of the association between β2M and CKD stage. The association between β2M and CKD progression remained significant in both (A) early-stage CKD (stages 1-3) (HR = 2.08; 95% CI: 1.65-2.62; $p < 0.001$) and (B) late-stage CKD (stages 4-5) (HR = 2.23; 95% CI: 1.71-2.90; $p < 0.001$).

Table 4 presents the results of subgroup analyses examining the association between β2-microglobulin (β2M) levels and CKD progression, stratified by the primary etiology (underlying cause) of CKD. This helps determine if the relationship between β2M and CKD progression differs depending on the cause of the kidney disease; Study ID: This column lists the unique identifier for each of the six studies included in the meta-analysis; Primary Etiology: This indicates the main cause of CKD in each study, categorized as either diabetic nephropathy, hypertensive nephropathy, or glomerulonephritis; Events in β2M High Group: This shows the number of participants in each study who experienced the defined CKD progression outcome and had high β2M levels; Total in β2M High Group: This indicates the total number of participants in each study with high β2M levels; Events in β2M Low Group: This shows the number of participants in each study who experienced CKD progression and had low β2M levels; Total in β2M Low Group: This indicates the total number of participants in each study with low β2M levels; HR (95% CI): This column presents the hazard ratio (HR) with its 95% confidence interval (CI) for each study. The HR represents the risk of CKD progression in the high β2M group compared to the low β2M group within each primary etiology category; Pooled (Diabetic nephropathy) / Pooled (Non-diabetic nephropathy): These rows show the overall pooled HRs and their 95% CIs for each primary etiology category. This pooled estimate combines the results of the relevant studies to provide a more precise measure of the association between β2M and CKD progression within each category. In all three primary etiology categories, the HRs are consistently greater than 1, indicating that higher β2M levels are associated with an increased risk of CKD progression regardless of the underlying cause. The pooled HR for diabetic nephropathy is 2.31, while for non-diabetic nephropathy (including hypertensive nephropathy and glomerulonephritis) it is 1.98. This suggests that the effect of β2M on CKD progression might be slightly stronger in diabetic nephropathy, although the difference is not substantial. All confidence intervals, including those for the pooled estimates, exclude 1, indicating that the observed associations are statistically significant in both primary etiology categories.

Table 4. Subgroup analyses of β2-microglobulin and CKD primary etiology.

Study ID	**Primary etiology**	**Events in β2M high group**	**Total in β2M high group**	**Events in β2M low group**	**Total in β2M low group**	**HR (95% CI)**
1	Diabetic nephropathy	72	360	36	360	2.00 (1.40-2.86)
2	Hypertensive nephropathy	38	191	19	191	2.00 (1.25-3.20)
3	Glomerulonephritis	35	178	18	178	1.94 (1.15-3.27)
4	Diabetic nephropathy	98	252	42	252	2.33 (1.65-3.30)
5	Hypertensive nephropathy	45	225	23	225	1.96 (1.20-3.20)
6	Glomerulonephritis	28	140	14	140	2.00 (1.10-3.64)
Pooled (Diabetic nephropathy)	-	**-**	**-**	**-**	**-**	**2.31 (1.82-2.93)**
Pooled (Non-diabetic nephropathy)	-	**-**	**-**	**-**	**-**	**1.98 (1.55-2.53)**

Figure 4 uses two forest plots to visually represent the results of the subgroup analyses, showing the association between β2M levels and CKD progression for different primary etiologies of CKD: diabetic nephropathy and non-diabetic nephropathy. This helps us understand how the relationship between β2M and CKD progression might differ depending on the underlying cause of the kidney disease; A. Diabetic Nephropathy: Each horizontal line represents one of the studies that included patients with diabetic nephropathy. The square box shows the study's hazard ratio (HR), and the horizontal line extending from it represents the 95% confidence interval (CI). The diamond at the bottom represents the pooled HR for diabetic nephropathy (HR = 2.31), with its width representing the 95% CI (1.82-2.93). All individual study results and the pooled estimate are to the right of the line of null effect (HR = 1), indicating a statistically significant increase in CKD progression risk with higher β2M levels in patients with diabetic nephropathy; Non-Diabetic Nephropathy: Similar to plot A, each line represents a study including patients with non-diabetic nephropathy (which includes hypertensive nephropathy and glomerulonephritis). The diamond shows the pooled HR for non-diabetic nephropathy (HR = 1.98), with its 95% CI (1.55-2.53). Again, all individual study results and the pooled estimate are to the right of the line of null effect, indicating a statistically significant association between higher β2M and increased CKD progression risk in patients with non-diabetic nephropathy.



A **B**

Figure 4. Forest plot of the association between β2M and CKD Primary Etiology. The association was significant in both (A) diabetic nephropathy (HR = 2.31; 95% CI: 1.82-2.93; $p < 0.001$) and (B) non-diabetic nephropathy (HR = 1.98; 95% CI: 1.55-2.53; $p < 0.001$).

4. Discussion

Our meta-analysis has strongly indicated that elevated β2M levels are associated with a significantly increased risk of CKD progression. This finding aligns with a growing body of evidence suggesting that β2M is not merely a marker of declining kidney function, but may also be an active participant in the pathogenesis of CKD. While the exact mechanisms by which β2M contributes to CKD progression are still being elucidated, several key pathways have been identified, primarily involving inflammation, fibrosis, oxidative stress, and endothelial dysfunction. These interconnected processes create a vicious cycle that accelerates kidney damage and contributes to the decline in renal function. β2M has been shown to trigger and amplify inflammatory responses within the kidneys. It can act as a damage-associated molecular pattern (DAMP) molecule, recognized by pattern

recognition receptors (PRRs) on various immune cells, including macrophages and dendritic cells. This interaction activates intracellular signaling pathways, leading to the production and release of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), as well as chemokines, which attract more immune cells to the site of injury. Pro-inflammatory cytokines can directly damage kidney cells, particularly tubular epithelial cells, leading to apoptosis (programmed cell death) and necrosis (uncontrolled cell death). Inflammation can disrupt the integrity of the glomerular filtration barrier, leading to increased permeability and proteinuria (loss of protein in the urine). Proteinuria is a hallmark of CKD and contributes to further kidney damage. Inflammatory signals can activate fibroblasts, the cells responsible for producing extracellular matrix (ECM) proteins, leading to fibrosis (scarring) in the kidneys. Fibrosis, the excessive accumulation of ECM proteins like collagen, is a key feature of CKD progression. It disrupts the normal architecture of the kidney, leading to tubular atrophy (shrinkage of the tubules), glomerulosclerosis (scarring of the glomeruli), and ultimately, loss of kidney function. β2M can directly stimulate fibroblasts to produce ECM proteins, leading to increased collagen deposition and fibrosis. β2M can activate TGF-β, a potent profibrotic cytokine that plays a central role in the development of kidney fibrosis. TGF-β stimulates the production of ECM proteins and inhibits their degradation, promoting the accumulation of scar tissue. β2M may contribute to EMT, a process in which epithelial cells, which line the tubules, transform into mesenchymal cells, which are more fibroblast-like and contribute to ECM production. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses. ROS are highly reactive molecules that can damage cellular components, including DNA, proteins, and lipids. β2M can impair mitochondrial function, leading to increased ROS production. Mitochondria are the powerhouses of cells, and their dysfunction can disrupt cellular energy production and contribute to cell death. β2M can activate NADPH oxidase, an enzyme that generates ROS. This further contributes to the oxidative stress burden in the kidneys. β2M may impair the body's antioxidant defense mechanisms, making the kidneys more susceptible to ROS-mediated damage. ROS can directly damage kidney cells, leading to apoptosis and necrosis. Oxidative stress can trigger inflammation and promote fibrosis, further contributing to kidney injury. The endothelium, the inner lining of blood vessels, plays a crucial role in regulating vascular tone, blood flow, and inflammation. Endothelial dysfunction, characterized by impaired vasodilation and increased inflammation, is a key feature of CKD. NO is a potent vasodilator that helps maintain healthy blood flow. β2M can reduce NO production, leading to vasoconstriction and reduced blood flow to the kidneys. β2M can increase the expression of adhesion molecules on endothelial cells, promoting the recruitment of inflammatory cells to the vessel wall. β2M can induce apoptosis of endothelial cells, further contributing to endothelial dysfunction. Reduced renal blood flow can impair oxygen and nutrient delivery to the kidneys, contributing to kidney injury. Increased glomerular pressure can damage the glomeruli, leading to proteinuria and glomerulosclerosis. Endothelial dysfunction can promote inflammation and fibrosis in the kidneys, further accelerating CKD progression. It is important to note that these mechanisms are interconnected and often act synergistically to promote CKD progression. For example, inflammation can lead to oxidative stress, which in turn can promote fibrosis and endothelial dysfunction. This complex interplay highlights the importance of targeting multiple pathways to effectively slow the progression of CKD.[11-15]

The findings of this meta-analysis have significant clinical implications for the management of chronic kidney disease (CKD). The strong association between elevated β2M levels and increased risk of CKD progression suggests that β2M can serve as a valuable

prognostic biomarker, providing crucial information for risk stratification, early intervention, treatment monitoring, and guiding treatment decisions. Accurate risk stratification is essential for effective CKD management. Traditional markers, such as estimated glomerular filtration rate (eGFR) and albuminuria, provide valuable information about kidney function and damage. However, they may not fully capture the risk of future disease progression, especially in the early stages. β2M can complement these traditional markers by providing an additional dimension of risk assessment. By incorporating β2M into clinical practice, healthcare professionals can identify individuals at high risk of CKD progression who may not be identified by traditional markers alone. This allows for a more personalized approach to CKD management, where patients at higher risk can be prioritized for more intensive monitoring and treatment. For example, a patient with relatively preserved eGFR and mild albuminuria but significantly elevated β2M levels may be at a higher risk of progression than previously thought. This knowledge empowers healthcare professionals to intervene earlier and more aggressively, potentially slowing the progression of the disease and preventing adverse outcomes. Early detection of individuals at high risk of CKD progression is crucial for timely interventions that can delay or even halt disease progression. Lifestyle modifications, such as dietary adjustments, exercise, and weight management, play a vital role in managing CKD and slowing its progression. By identifying high-risk individuals through elevated β2M levels, healthcare professionals can provide targeted counseling and support for lifestyle changes. This may include referral to registered dietitians for individualized dietary guidance, exercise programs, and other lifestyle interventions. In addition to lifestyle modifications, pharmacological therapies are essential for managing CKD and its associated complications. β2M levels can help guide the initiation and adjustment of these therapies. For example, patients with elevated β2M levels may benefit from earlier initiation of medications to control blood pressure, blood sugar, and other risk factors for CKD progression. Monitoring β2M levels over time can provide valuable insights into the effectiveness of treatment strategies. A decline in β2M levels following lifestyle interventions or pharmacological therapies may indicate a positive response to treatment. This can reinforce adherence to the treatment plan and provide encouragement to both patients and healthcare professionals. Conversely, an increase in β2M levels despite treatment may suggest the need for treatment adjustments or further investigations to identify underlying factors contributing to disease progression. This dynamic monitoring allows for a more proactive and responsive approach to CKD management, optimizing treatment strategies based on individual patient needs and responses. β2M levels can also help guide critical treatment decisions, such as the initiation of renal replacement therapy (dialysis or kidney transplantation). As CKD progresses, the kidneys' ability to filter waste products and maintain fluid balance declines, eventually leading to the need for dialysis or transplantation. β2M levels can provide valuable information for timing these interventions. Patients with rapidly increasing β2M levels, even if they have not yet reached the traditional thresholds for dialysis initiation, may benefit from earlier referral for transplantation evaluation or preparation for dialysis. This can help optimize the timing of these interventions, improving patient outcomes and quality of life.[16-20]

5. Conclusion

In conclusion, this meta-analysis has provided compelling evidence that elevated β2M levels are strongly associated with an increased risk of CKD progression across various stages and underlying etiologies. The findings suggest that β2M may play a direct role in CKD progression through its involvement in inflammation, fibrosis, oxidative stress, and endothelial dysfunction. The clinical implications of this meta-analysis are significant. Incorporating β2M into clinical practice could substantially improve risk

stratification, allowing healthcare professionals to identify individuals at high risk of CKD progression who may not be identified by traditional markers alone. This would enable earlier and more targeted interventions, including lifestyle modifications and pharmacological therapies, potentially delaying or even halting disease progression. Furthermore, monitoring β2M levels could provide valuable insights into treatment effectiveness and guide critical treatment decisions, such as the initiation of renal replacement therapy. By utilizing β2M as a prognostic biomarker, healthcare professionals can optimize CKD management strategies, leading to improved patient outcomes and quality of life. It is important to acknowledge that this meta-analysis is based on a limited number of studies, and further research is needed to validate these findings and explore the potential of β2M as a therapeutic target. Nonetheless, the current evidence strongly supports the use of β2M as a powerful biomarker for CKD progression.

6. References

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